

What is claimed is:

1. A method for detecting the presence or absence of at least one target nucleic acid sequence in a sample comprising:

5 forming a ligation reaction composition comprising the sample, and a ligation probe set for each target nucleic acid sequence, the probe set comprising (a) at least one first probe, comprising a target-specific portion and a 5' primer-specific portion, wherein the 5' primer-specific portion comprises a sequence, and (b) at least one second probe, comprising a target-specific portion and a 3'

10 primer-specific portion, wherein the 3' primer-specific portion comprises a sequence, wherein the probes in each set are suitable for ligation together when hybridized adjacent to one another on a complementary target sequence;

forming a test composition by subjecting the ligation reaction composition to at least one cycle of ligation, wherein adjacently hybridizing complementary

15 probes are ligated to one another to form a ligation product comprising the 5' primer-specific portion, the target-specific portions, and the 3' primer-specific portion;

forming at least one amplification reaction composition comprising:

at least a portion of the test composition;

20 a polymerase;

a double-stranded-dependent specific label, wherein the double-stranded-dependent label has a first detectable signal value when

the double-stranded-dependent label is not exposed to double-stranded nucleic acid; and

at least one primer set, the primer set comprising (i) at least one first primer comprising the sequence of the 5' primer-specific portion of the ligation product, and (ii) at least one second primer comprising a sequence complementary to the sequence of the 3' primer-specific portion of the ligation product;

subjecting the at least one amplification reaction composition to at least one amplification reaction; and

detecting a second detectable signal value at least one of during and after the at least one amplification reaction, wherein a threshold difference between the first detectable signal value and the second detectable signal value indicates the presence of the target nucleic acid sequence, and wherein no threshold difference between the first detectable signal value and the second detectable signal value indicates the absence of the target nucleic acid sequence.

2. The method of claim 1, wherein:

the ligation reaction composition comprises:

at least two different probe sets for detecting at least two different target

nucleic acid sequences, and wherein

a first probe set comprises (a) at least one first probe, comprising a target-specific portion that hybridizes to a first portion of a first target nucleic acid sequence and a 5' primer-specific portion, wherein the 5' primer-specific portion

comprises a sequence and (b) at least one second probe, comprising a target-specific portion that hybridizes to a second portion of the first target nucleic acid sequence and a 3' primer-specific portion, wherein the 3' primer-specific portion comprises a sequence; and

5           a second probe set comprises (a) at least one first probe, comprising a target-specific portion that hybridizes to a first portion of a second target nucleic acid sequence, and a 5' primer-specific portion, wherein the 5' primer-specific portion comprises a sequence, and (b) at least one second probe, comprising a target-specific portion that hybridizes to a second portion of the second target  
10   nucleic acid sequence, and a 3' primer-specific portion, wherein the 3' primer-specific portion comprises a sequence;

          wherein the sequence of the 5' primer-specific portion of the first probe of the first probe set is different from the sequence of the 5' primer-specific portion of the first probe of the second probe set and wherein the first target nucleic acid  
15   sequence is different from the second target nucleic acid sequence.

3.       The method of claim 2:

          wherein the forming of the at least one amplification reaction composition comprises forming at least two amplification reaction compositions comprising:

20       a first amplification reaction composition comprising:

          at least a portion of the test composition;

          a polymerase;

a double-stranded-dependent specific label, wherein the double-stranded-dependent label has a first detectable signal value when the double-stranded-dependent label is not exposed to double-stranded nucleic acid; and

5 a first primer set, the first primer set comprising (i) at least one first primer comprising the sequence of the 5' primer-specific portion of the at least one first probe of the first probe set, and (ii) at least one second primer comprising a sequence complementary to the sequence of the 3' primer-specific portion of the at least one

10 second probe of the first probe set; and

a second amplification reaction composition comprising:

at least a portion of the test composition;

a polymerase;

a double-stranded-dependent label, wherein the double-stranded-dependent label has a first detectable signal value when the

15 double-stranded-dependent label is not exposed to double-stranded nucleic acid; and

a second primer set, the second primer set comprising (i) at least one first primer comprising the sequence of the 5' primer-specific portion of the at least one first probe of the second probe set, and

20 (ii) at least one second primer comprising a sequence complementary to the sequence of the 3' primer-specific portion of the at least one second probe of the second probe set;

and wherein each of the at least two amplification reaction compositions are subjected to at least one amplification reaction;

and wherein the detecting comprises:

- detecting a second detectable signal value at least one of during and after
- 5 the at least one amplification reaction of the first amplification reaction composition, wherein a threshold difference between the first detectable signal value and the second detectable signal value of the at least one amplification reaction of the first amplification reaction composition indicates the presence of the first target nucleic acid sequence, and wherein no threshold difference
- 10 between the first detectable signal value and the second detectable signal value of the at least one amplification reaction of the first amplification reaction composition indicates the absence of the first target nucleic acid sequence; and
- detecting a second detectable signal value at least one of during and after
- the at least one amplification reaction of the second amplification reaction
- 15 composition, wherein a threshold difference between the first detectable signal value and the second detectable signal value of the amplification reaction of the second amplification reaction composition indicates the presence of the second target nucleic acid sequence, and wherein no threshold difference between the first detectable signal value and the second detectable signal value of the at least
- 20 one amplification reaction of the second amplification reaction composition indicates the absence of the second target nucleic acid sequence.

4. The method of claim 1, wherein:

the ligation reaction composition comprises:

at least two different probe sets for detecting at least two different target nucleic acid sequences, and wherein

a first probe set comprises (a) at least one first probe, comprising a target-specific portion that hybridizes to a first portion of a first target nucleic acid sequence and a 5' primer-specific portion, wherein the 5' primer-specific portion comprises a sequence and (b) at least one second probe, comprising a target-specific portion that hybridizes to a second portion of the first target nucleic acid sequence and a 3' primer-specific portion, wherein the 3' primer-specific portion comprises a sequence; and

a second probe set comprises (a) at least one first probe, comprising a target-specific portion that hybridizes to a first portion of a second target nucleic acid sequence, and a 5' primer-specific portion, wherein the 5' primer-specific portion comprises a sequence, and (b) at least one second probe, comprising a target-specific portion that hybridizes to a second portion of the second target nucleic acid sequence, and a 3' primer-specific portion, wherein the 3' primer-specific portion comprises a sequence;

wherein the sequence of the 3' primer-specific portion of the second probe of the first probe set is different from the sequence of the 3' primer-specific portion of the second probe of the second probe set and wherein the first target nucleic acid sequence is different from the second target nucleic acid sequence.

5. The method of claim 4:

wherein the forming of the at least one amplification reaction composition comprises forming at least two amplification reaction compositions comprising:

a first amplification reaction composition comprising:

at least a portion of the test composition;

5 a polymerase;

a double-stranded-dependent label, wherein the double-stranded-dependent label has a first detectable signal value when the double-stranded-dependent label is not exposed to double-stranded nucleic acid; and

10 a first primer set, the first primer set comprising (i) at least one first primer comprising the sequence of the 5' primer-specific portion of the at least one first probe of the first probe set, and (ii) at least one second primer comprising a sequence complementary to the sequence of the 3' primer-specific portion of the at least one  
15 second probe of the first probe set; and

a second amplification reaction composition comprising:

at least a portion of the test composition;

a polymerase;

20 a double-stranded-dependent label, wherein the double-stranded-dependent label has a first detectable signal value when the double-stranded-dependent label is not exposed to double-stranded nucleic acid; and

a second primer set, the second primer set comprising (i) at least one first primer comprising the sequence of the 5' primer-specific portion of the at least one first probe of the second probe set, and (ii) at least one second primer comprising a sequence complementary to the sequence of the 3' primer-specific portion of the at least one second probe of the second probe set;

5                   and wherein each of the at least two amplification reaction compositions are subjected to at least one amplification reaction;

                  and wherein the detecting comprises:

10           detecting a second detectable signal value at least one of during and after the at least one amplification reaction of the first amplification reaction composition, wherein a threshold difference between the first detectable signal value and the second detectable signal value of the at least one amplification reaction of the first amplification reaction composition indicates the presence of

15   the first target nucleic acid sequence, and wherein no threshold difference between the first detectable signal value and the second detectable signal value of the at least one amplification reaction of the first amplification reaction composition indicates the absence of the first target nucleic acid sequence; and

                  detecting a second detectable signal value at least one of during and after

20   the at least one amplification reaction of the second amplification reaction composition, wherein a threshold difference between the first detectable signal value and the second detectable signal value of the at least one amplification reaction of the second amplification reaction composition indicates the presence



of the second target nucleic acid sequence, and wherein no threshold difference between the first detectable signal value and the second detectable signal value of the at least one amplification reaction of the second amplification reaction composition indicates the absence of the second target nucleic acid sequence.

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6. The method of any one of claims 2 to 5, wherein the first target nucleic acid sequence and the second target nucleic acid sequence have different nucleotides at a given locus.

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7. A method for detecting the presence or absence of at least one target nucleic acid sequence in a sample comprising:

forming a ligation reaction composition comprising the sample, and a ligation probe set for each target nucleic acid sequence, the probe set comprising (a) at least one first probe, comprising a target-specific portion and a 5' primer-specific portion, wherein the 5' primer-specific portion comprises a sequence, and (b) at least one second probe, comprising a target-specific portion and a 3' primer-specific portion, wherein the 3' primer-specific portion comprises a sequence, wherein the probes in each set are suitable for ligation together when hybridized adjacent to one another on a complementary target sequence;

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forming a test composition by subjecting the ligation reaction composition to at least one cycle of ligation, wherein adjacently hybridizing complementary probes are ligated to one another to form a ligation product comprising the 5'

primer-specific portion, the target-specific portions, and the 3' primer-specific portion;

forming at least one amplification reaction composition comprising:

at least a portion of the test composition,

5 a polymerase,

a double-stranded-dependent label; and

at least one primer set, the primer set comprising (i) at least one

first primer comprising the sequence of the 5' primer-specific

portion of the ligation product, and (ii) at least one second primer

10 comprising a sequence complementary to the sequence of the 3' primer-specific portion of the ligation product;

subjecting the at least one amplification reaction composition to at least one amplification reaction; and

detecting the presence or absence of the target nucleic acid sequence by

15 monitoring a signal at least one of during and after the at least one amplification reaction.

8. The method of claim 7:

wherein the detecting comprises determining a threshold cycle ( $C_t$ )

20 value from the monitoring of the signal.

9. The method of claim 7:

wherein the detecting comprises determining a threshold time ( $T_t$ ) value from the monitoring of the signal.

10. The method of claim 7, wherein:

5 the ligation reaction composition comprises:

at least two different probe sets for detecting at least two different target nucleic acid sequences, and wherein

a first probe set comprises (a) at least one first probe, comprising a target-specific portion that hybridizes to a first portion of a first target nucleic acid  
10 sequence and a 5' primer-specific portion, wherein the 5' primer-specific portion comprises a sequence and (b) at least one second probe, comprising a target-specific portion that hybridizes to a second portion of the first target nucleic acid sequence and a 3' primer-specific portion, wherein the 3' primer-specific portion comprises a sequence; and

15 a second probe set comprises (a) at least one first probe, comprising a target-specific portion that hybridizes to a first portion of a second target nucleic acid sequence, and a 5' primer-specific portion, wherein the 5' primer-specific portion comprises a sequence, and (b) at least one second probe, comprising a target-specific portion that hybridizes to a second portion of the second target  
20 nucleic acid sequence, and a 3' primer-specific portion, wherein the 3' primer-specific portion comprises a sequence;

wherein the sequence of the 5' primer-specific portion of the first probe of the first probe set is different from the sequence of the 5' primer-specific portion

of the first probe of the second probe set and wherein the first target nucleic acid sequence is different from the second target nucleic acid sequence.

11. The method of claim 10:

5 wherein the forming of the at least one amplification reaction composition comprises forming at least two amplification reaction compositions comprising:

a first amplification reaction composition comprising:

at least a portion of the test composition;

a polymerase;

10 a double-stranded-dependent label; and

a first primer set, the first primer set comprising (i) at least one first primer comprising the sequence of the 5' primer-specific portion of

the at least one first probe of the first probe set, and (ii) at least one

second primer comprising a sequence complementary to the

15 sequence of the 3' primer-specific portion of the at least one

second probe of the first probe set; and

a second amplification reaction composition comprising:

at least a portion of the test composition;

a polymerase;

20 a double-stranded-dependent label; and

a second primer set, the second primer set comprising (i) at least one first primer comprising the sequence of the 5' primer-specific

portion of the at least one first probe of the second probe set, and

(ii) at least one second primer comprising a sequence complementary to the sequence of the 3' primer-specific portion of the at least one second probe of the second probe set; and wherein each of the at least two amplification reaction compositions are subjected to at least one amplification reaction; and wherein the detecting comprises: detecting the presence or absence of the first target nucleic acid sequence by monitoring a signal at least one of during and after the at least one amplification reaction of the first amplification reaction composition; and  
10 detecting the presence or absence of the second target nucleic acid sequence by monitoring a signal at least one of during and after the at least one amplification reaction of the second amplification reaction composition.

12. The method of claim 11, wherein  
15 the detecting of the presence or absence of the first target nucleic acid sequence comprises determining a first  $C_t$  value from the monitoring of the signal of the at least one amplification reaction of the first amplification reaction composition; and  
the detecting of the presence or absence of the second target nucleic acid  
20 sequence comprises determining a second  $C_t$  value from the monitoring of the signal of the at least one amplification reaction of the second amplification reaction composition.

13. The method of claim 12, wherein the detecting of the presence or absence of the first target nucleic acid sequence and the second target nucleic acid sequence comprises comparing the first  $C_t$  value to the second  $C_t$  value.

5 14. The method of claim 12, wherein the first target nucleic acid sequence and the second target nucleic acid sequence have different nucleotides at a given locus, and the detecting of the presence or absence of the first target nucleic acid sequence and the second target nucleic acid sequence comprises comparing the first  $C_t$  value to the second  $C_t$  value.

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15. The method of claim 11, wherein the detecting of the presence or absence of the first target nucleic acid sequence comprises determining a first  $T_1$  value from the monitoring of the signal of the at least one amplification reaction of the first amplification reaction

15 composition; and

the detecting of the presence or absence of the second target nucleic acid sequence comprises determining a second  $T_1$  value from the monitoring of the signal of the at least one amplification reaction of the second amplification reaction composition.

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16. The method of claim 15, wherein the detecting of the presence or absence of the first target nucleic acid sequence and the second target nucleic acid sequence comprises comparing the first  $T_1$  value to the second  $T_1$  value.

17. The method of claim 15, wherein the first target nucleic acid sequence and the second target nucleic acid sequence have different nucleotides at a given locus, and the detecting of the presence or absence of the first target nucleic acid sequence and the second target nucleic acid sequence comprises comparing the first  $T_1$  value to the second  $T_1$  value.

18. The method of claim 7, wherein:  
the ligation reaction composition comprises:  
at least two different probe sets for detecting at least two different target nucleic acid sequences, and wherein  
a first probe set comprises (a) at least one first probe, comprising a target-specific portion that hybridizes to a first portion of a first target nucleic acid sequence and a 5' primer-specific portion, wherein the 5' primer-specific portion comprises a sequence and (b) at least one second probe, comprising a target-specific portion that hybridizes to a second portion of the first target nucleic acid sequence and a 3' primer-specific portion, wherein the 3' primer-specific portion comprises a sequence; and  
a second probe set comprises (a) at least one first probe, comprising a target-specific portion that hybridizes to a first portion of a second target nucleic acid sequence, and a 5' primer-specific portion, wherein the 5' primer-specific portion comprises a sequence, and (b) at least one second probe, comprising a target-specific portion that hybridizes to a second portion of the second target

nucleic acid sequence, and a 3' primer-specific portion, wherein the 3' primer-specific portion comprises a sequence;

wherein the sequence of the 3' primer-specific portion of the second probe of the first probe set is different from the sequence of the 3' primer-specific portion of the second probe of the second probe set and wherein the first target nucleic acid sequence is different from the second target nucleic acid sequence.

19. The method of claim 18:

wherein the forming of the at least one amplification reaction composition comprises forming at least two amplification reaction compositions comprising:

a first amplification reaction composition comprising:

at least a portion of the test composition;

a polymerase;

a double-stranded-dependent; and

a first primer set, the first primer set comprising (i) at least one first primer comprising the sequence of the 5' primer-specific portion of the at least one first probe of the first probe set, and (ii) at least one second primer comprising a sequence complementary to the sequence of the 3' primer-specific portion of the at least one second probe of the first probe set; and

a second amplification reaction composition comprising:

at least a portion of the test composition;

a polymerase;



a double-stranded-dependent label; and

a second primer set, the second primer set comprising (i) at least

one first primer comprising the sequence of the 5' primer-specific

portion of the at least one first probe of the second probe set, and

5 (ii) at least one second primer comprising a sequence

complementary to the sequence of the 3' primer-specific portion of

the at least one second probe of the second probe set;

and wherein each of the at least two amplification reaction compositions

are subjected to at least one amplification reaction;

10 and wherein the detecting comprises:

detecting the presence or absence of the first target nucleic acid sequence

by monitoring a signal at least one of during and after the at least one

amplification reaction of the first amplification reaction composition; and

detecting the presence or absence of the second target nucleic acid

15 sequence by monitoring a signal at least one of during and after the at least one

amplification reaction of the second amplification reaction composition.

20. The method of claim 19, wherein

the detecting of the presence or absence of the first target nucleic acid

20 sequence comprises determining a first  $C_i$  value from the monitoring of the signal

of the at least one amplification reaction of the first amplification reaction

composition; and

the detecting of the presence or absence of the second target nucleic acid sequence comprises determining a second  $C_i$  value from the monitoring of the signal of the at least one amplification reaction of the second amplification reaction composition.

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21. The method of claim 20, wherein the detecting of the presence or absence of the first target nucleic acid sequence and the second target nucleic acid sequence comprises comparing the first  $C_i$  value to the second  $C_i$  value.

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22. The method of claim 20, wherein the first target nucleic acid sequence and the second target nucleic acid sequence have different nucleotides at a given locus, and the detecting of the presence or absence of the first target nucleic acid sequence and the second target nucleic acid sequence comprises comparing the first  $C_i$  value to the second  $C_i$  value.

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23. The method of claim 19, wherein

the detecting of the presence or absence of the first target nucleic acid sequence comprises determining a first  $T_i$  value from the monitoring of the signal of the at least one amplification reaction of the first amplification reaction

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composition; and

the detecting of the presence or absence of the second target nucleic acid sequence comprises determining a second  $T_i$  value from the monitoring of the

signal of the at least one amplification reaction of the second amplification reaction composition.

24. The method of claim 23, wherein the detecting of the presence or  
5 absence of the first target nucleic acid sequence and the second target nucleic acid sequence comprises comparing the first  $T_1$  value to the second  $T_1$  value.

25. The method of claim 23, wherein the first target nucleic acid  
sequence and the second target nucleic acid sequence have different  
10 nucleotides at a given locus, and the detecting of the presence or absence of the first target nucleic acid sequence and the second target nucleic acid sequence comprises comparing the first  $T_1$  value to the second  $T_1$  value.

26. The method of any one of claims 10 to 12, 15, 18 to 20, and 23,  
15 wherein the first target nucleic acid sequence and the second target nucleic acid sequence have different nucleotides at a given locus.

27. A method for detecting the presence or absence of at least one target nucleic acid sequence in a sample comprising:

20 (a) forming at least one reaction composition comprising:

the sample;

a ligation probe set for the target nucleic acid sequence, the probe set comprising (a) at least one first probe, comprising a target-specific

portion and a 5' primer-specific portion, wherein the 5' primer-specific portion comprises a sequence and (b) at least one second probe, comprising a target-specific portion and a 3' primer-specific portion, wherein the 3' primer-specific portion comprises a sequence, wherein the probes in each set are suitable for ligation together when hybridized adjacent to one another on a complementary target sequence;

a polymerase;

a double-stranded-dependent label, wherein the double-stranded-dependent label has a first detectable signal value when the double-stranded-dependent label is not exposed to double-stranded nucleic acid; and

at least one primer set, the primer set comprising (i) at least one first primer comprising the sequence of the 5' primer-specific portion of the ligation product, and (ii) at least one second primer comprising a sequence complementary to the sequence of the 3' primer-specific portion of the ligation product;

(b) subjecting the reaction composition to at least one cycle of ligation, wherein adjacently hybridizing complementary probes are ligated to one another to form a ligation product comprising the 5' primer-specific portion, the target-specific portions, and the 3' primer-specific portion;

(c) after the at least one cycle of ligation, subjecting the reaction composition to at least one amplification reaction; and

(d) detecting a second detectable signal value at least one of during and after the at least one amplification reaction, wherein a threshold difference between the first detectable signal value and the second detectable signal value indicates the presence of the target nucleic acid sequence, and wherein no threshold difference between the first detectable signal value and the second detectable signal value indicates the absence of the target nucleic acid sequence.

28. The method of claim 27:

wherein the forming of the at least one reaction composition comprises forming at least two reaction compositions for detecting at least two different target nucleic acid sequences, the at least two reaction compositions comprising:

a first reaction composition comprising:

a first probe set comprises (a) at least one first probe, comprising a target-specific portion that hybridizes to a first portion of a first target nucleic acid sequence and a 5' primer-specific portion, wherein the 5' primer-specific portion comprises a sequence and (b) at least one second probe, comprising a target-specific portion that hybridizes to a second portion of the first target nucleic acid sequence and a 3' primer-specific portion, wherein the 3' primer-specific portion comprises a sequence;

a polymerase;

a double-stranded-dependent label, wherein the double-stranded-dependent label has a first detectable signal value when the double-stranded-dependent label is not exposed to double-stranded nucleic acid; and

5 a first primer set, the first primer set comprising (i) at least one first primer comprising the sequence of the 5' primer-specific portion of the at least one first probe of the first probe set, and (ii) at least one second primer comprising a sequence complementary to the sequence of the 3' primer-specific portion of the at least one  
10 second probe of the first probe set; and

a second amplification reaction composition comprising:

a second probe set comprises (a) at least one first probe, comprising a target-specific portion that hybridizes to a first portion of a second target nucleic acid sequence, and a 5' primer-specific  
15 portion, wherein the 5' primer-specific portion comprises a sequence, and (b) at least one second probe, comprising a target-specific portion that hybridizes to a second portion of the second target nucleic acid sequence, and a 3' primer-specific portion, wherein the 3' primer-specific portion comprises a sequence;

20 a polymerase;

a double-stranded-dependent label, wherein the double-stranded-dependent label has a first detectable signal value when the

double-stranded-dependent label is not exposed to double-stranded nucleic acid; and

a second primer set, the second primer set comprising (i) at least one first primer comprising the sequence of the 5' primer-specific portion of the at least one first probe of the second probe set, and

5 (ii) at least one second primer comprising a sequence complementary to the sequence of the 3' primer-specific portion of the at least one second probe of the second probe set;

wherein the first target nucleic acid sequence is different from the second

10 target nucleic acid sequence; and

wherein each of the at least two amplification reaction compositions are subjected to at least one amplification reaction;

and wherein the detecting comprises:

detecting a second detectable signal value at least one of during and after

15 the at least one amplification reaction of the first amplification reaction composition, wherein a threshold difference between the first detectable signal value and the second detectable signal value of the at least one amplification reaction of the first amplification reaction composition indicates the presence of the first target nucleic acid sequence, and wherein no threshold difference

20 between the first detectable signal value and the second detectable signal value of the at least one amplification reaction of the first amplification reaction composition indicates the absence of the first target nucleic acid sequence; and

detecting a second detectable signal value at least one of during and after  
the at least one amplification reaction of the second amplification reaction  
composition, wherein a threshold difference between the first detectable signal  
value and the second detectable signal value of the at least one amplification  
5 reaction of the second amplification reaction composition indicates the presence  
of the second target nucleic acid sequence, and wherein no threshold difference  
between the first detectable signal value and the second detectable signal value  
of the at least one amplification reaction of the second amplification reaction  
composition indicates the absence of the second target nucleic acid sequence.

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29. The method of claim 28, wherein the sequence of the 5' primer-  
specific portion of the first probe of the first probe set is the same as the  
sequence of the 5' primer-specific portion of the first probe of the second probe  
set.

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30. The method of claim 29, wherein the sequence of the 3' primer-  
specific portion of the first probe of the first probe set is the same as the  
sequence of the 3' primer-specific portion of the first probe of the second probe  
set.

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31. The method of claim 28, wherein the sequence of the 3' primer-  
specific portion of the first probe of the first probe set is the same as the



sequence of the 3' primer-specific portion of the first probe of the second probe set.

32. The method of any one of claims 28 to 31, wherein the first target  
5 nucleic acid sequence and the second target nucleic acid sequence have  
different nucleotides at a given locus.

33. A method for detecting the presence or absence of at least one  
target nucleic acid sequence in a sample comprising:

10 (a) forming at least one reaction composition comprising:

the sample;

a ligation probe set for the target nucleic acid sequence, the probe  
set comprising (a) at least one first probe, comprising a target-specific  
portion and a 5' primer-specific portion, wherein the 5' primer-specific  
15 portion comprises a sequence and (b) at least one second probe,  
comprising a target-specific portion and a 3' primer-specific portion,  
wherein the 3' primer-specific portion comprises a sequence, wherein the  
probes in each set are suitable for ligation together when hybridized  
adjacent to one another on a complementary target sequence;

20 a polymerase;

a double-stranded-dependent label; and

at least one primer set, the primer set comprising (i) at least one  
first primer comprising the sequence of the 5' primer-specific portion of the

ligation product, and (ii) at least one second primer comprising a sequence complementary to the sequence of the 3' primer-specific portion of the ligation product;

(b) subjecting the reaction composition to at least one cycle of ligation, wherein adjacently hybridizing complementary probes are ligated to one another to form a ligation product comprising the 5' primer-specific portion, the target-specific portions, and the 3' primer-specific portion;

(c) after the at least one cycle of ligation, subjecting the reaction composition to at least one amplification reaction; and

(d) detecting the presence or absence of the target nucleic acid sequence by monitoring a signal at least one of during and after the at least one amplification reaction.

34. The method of claim 33:

wherein the detecting comprises determining a threshold cycle ( $C_t$ ) value from the monitoring of the signal.

35. The method of claim 33:

wherein the detecting comprises determining a threshold time ( $T_t$ ) value from the monitoring of the signal.

36. The method of claim 33:

wherein the forming of the at least one reaction composition comprises forming at least two reaction compositions for detecting at least two different target nucleic acid sequences, the at least two reaction compositions comprising:

a first reaction composition comprising:

- 5                   a first probe set comprises (a) at least one first probe, comprising a target-specific portion that hybridizes to a first portion of a first target nucleic acid sequence and a 5' primer-specific portion, wherein the 5' primer-specific portion comprises a sequence and (b) at least one second probe, comprising a target-specific portion
- 10                   that hybridizes to a second portion of the first target nucleic acid sequence and a 3' primer-specific portion, wherein the 3' primer-specific portion comprises a sequence;
- a polymerase;
- a double-stranded-dependent; and
- 15                   a first primer set, the first primer set comprising (i) at least one first primer comprising the sequence of the 5' primer-specific portion of the at least one first probe of the first probe set, and (ii) at least one second primer comprising a sequence complementary to the sequence of the 3' primer-specific portion of the at least one
- 20                   second probe of the first probe set; and

a second reaction composition comprising:

- a second probe set comprises (a) at least one first probe, comprising a target-specific portion that hybridizes to a first portion

of a second target nucleic acid sequence, and a 5' primer-specific portion, wherein the 5' primer-specific portion comprises a sequence, and (b) at least one second probe, comprising a target-specific portion that hybridizes to a second portion of the second target nucleic acid sequence, and a 3' primer-specific portion, wherein the 3' primer-specific portion comprises a sequence; a polymerase; a double-stranded-dependent label; and a second primer set, the second primer set comprising (i) at least one first primer comprising the sequence of the 5' primer-specific portion of the at least one first probe of the second probe set, and (ii) at least one second primer comprising a sequence complementary to the sequence of the 3' primer-specific portion of the at least one second probe of the second probe set; wherein the first target nucleic acid sequence is different from the second target nucleic acid sequence; and wherein each of the at least two reaction compositions are subjected to at least one amplification reaction; and wherein the detecting comprises: detecting the presence or absence of the first target nucleic acid sequence by monitoring a signal at least one of during and after the at least one amplification reaction of the first reaction composition; and

detecting the presence or absence of the second target nucleic acid sequence by monitoring a signal at least one of during and after the at least one amplification reaction of the second reaction composition.

5           37.    The method of claim 36, wherein

the detecting of the presence or absence of the first target nucleic acid sequence comprises determining a first  $C_t$  value from the monitoring of the signal of the at least one amplification reaction of the first reaction composition; and

10           the detecting of the presence or absence of the second target nucleic acid sequence comprises determining a second  $C_t$  value from the monitoring of the signal of the at least one amplification reaction of the second reaction composition.

15           38.    The method of claim 36, wherein the first target nucleic acid sequence and the second target nucleic acid sequence have different nucleotides at a given locus.

20           39.    The method of claim 37, wherein the first target nucleic acid sequence and the second target nucleic acid sequence have different nucleotides at a given locus.

40. The method of claim 37, wherein the detecting of the presence or absence of the first target nucleic acid sequence and the second target nucleic acid sequence comprises comparing the first  $C_i$  value to the second  $C_i$  value.

5           41. The method of claim 37, wherein the first target nucleic acid sequence and the second target nucleic acid sequence have different nucleotides at a given locus, and the detecting of the presence or absence of the first target nucleic acid sequence and the second target nucleic acid sequence comprises comparing the first  $C_i$  value to the second  $C_i$  value.

10           42. The method of claim 36, wherein  
the detecting of the presence or absence of the first target nucleic acid sequence comprises determining a first  $T_i$  value from the monitoring of the signal of the at least one amplification reaction of the first reaction composition; and  
15           the detecting of the presence or absence of the second target nucleic acid sequence comprises determining a second  $T_i$  value from the monitoring of the signal of the at least one amplification reaction of the second reaction composition.

20           43. The method of claim 42, wherein the first target nucleic acid sequence and the second target nucleic acid sequence have different nucleotides at a given locus.

44. The method of claim 42, wherein the detecting of the presence or absence of the first target nucleic acid sequence and the second target nucleic acid sequence comprises comparing the first  $T_1$  value to the second  $T_1$  value.

5

45. The method of claim 42, wherein the first target nucleic acid sequence and the second target nucleic acid sequence have different nucleotides at a given locus, and the detecting of the presence or absence of the first target nucleic acid sequence and the second target nucleic acid sequence  
10 comprises comparing the first  $T_1$  value to the second  $T_1$  value.

46. The method of any one of claims 36 to 45, wherein the sequence of the 5' primer-specific portion of the first probe of the first probe set is the same as the sequence of the 5' primer-specific portion of the first probe of the second  
15 probe set.

47. The method of claim 46, wherein the sequence of the 3' primer-specific portion of the first probe of the first probe set is the same as the sequence of the 3' primer-specific portion of the first probe of the second probe  
20 set.

48. The method of any one of claims 36 to 45, wherein the sequence of the 3' primer-specific portion of the first probe of the first probe set is the same as

the sequence of the 3' primer-specific portion of the first probe of the second probe set.

49. A kit for detecting at least one target nucleic acid sequence in a

5 sample comprising:

(a) a ligation probe set for each target nucleic acid sequence, the probe set comprising

(i) at least one first probe, comprising a target-specific portion, a 5' primer-specific portion, wherein the 5' primer-specific portion comprises a sequence,

10 and

(ii) at least one second probe, comprising a target-specific portion, a 3' primer-specific portion, wherein the 3' primer-specific portion comprises a sequence,

wherein the probes in each set are suitable for ligation together when  
15 hybridized adjacent to one another on a complementary target nucleic acid sequence; and

(b) a double-stranded-dependent label.

50. The kit of claim 49, further comprising at least one primer set

20 comprising (i) at least one first primer comprising the sequence of the 5' primer-specific portion of the at least one first probe, and (ii) at least one second primer comprising a sequence complementary to the sequence of the 3' primer-specific portion of the at least one second probe.



51. A method for detecting the presence or absence of at least one target nucleic acid sequence in a sample comprising:

forming a ligation reaction composition comprising the sample; a ligation probe set for each target nucleic acid sequence, the probe set comprising (a) at least one first probe, comprising a target-specific portion, and (b) at least one second probe, comprising a target-specific portion, wherein the probes in each set are suitable for ligation together when hybridized adjacent to one another on a complementary target sequence; and poly-deoxy-inosinic-deoxy-cytidylic acid;

forming a test composition by subjecting the ligation reaction composition to at least one cycle of ligation, wherein adjacently hybridizing complementary probes are ligated to one another to form a ligation product comprising the 5' primer-specific portion, the target-specific portions, and the 3' primer-specific portion; and

detecting the presence or absence of the ligation product to detect the presence or absence of the at least one target nucleic acid sequence.

52. A method for detecting the presence or absence of at least one target nucleic acid sequence in a sample comprising:

forming a ligation reaction composition comprising the sample, poly-deoxy-inosinic-deoxy-cytidylic acid, and a ligation probe set for each target nucleic acid sequence, the probe set comprising (a) at least one first probe, comprising a target-specific portion and a 5' primer-specific portion, wherein the 5' primer-specific portion comprises a sequence, and (b) at least one second

probe, comprising a target-specific portion and a 3' primer-specific portion,  
wherein the 3' primer-specific portion comprises a sequence, wherein the probes  
in each set are suitable for ligation together when hybridized adjacent to one  
another on a complementary target sequence;

5           forming a test composition by subjecting the ligation reaction composition  
to at least one cycle of ligation, wherein adjacently hybridizing complementary  
probes are ligated to one another to form a ligation product comprising the 5'  
primer-specific portion, the target-specific portions, and the 3' primer-specific  
portion;

10           forming at least one amplification reaction composition comprising:  
            at least a portion of the test composition;  
            a polymerase; and  
            at least one primer set, the primer set comprising (i) at least one  
            first primer comprising the sequence of the 5' primer-specific  
15           portion of the ligation product, and (ii) at least one second primer  
            comprising a sequence complementary to the sequence of the 3'  
            primer-specific portion of the ligation product;

            subjecting the at least one amplification reaction composition to at least  
one amplification reaction; and

20           detecting the presence or absence of the target nucleic acid sequence by  
detecting whether the at least one amplification reaction results in amplification  
product from ligation product.

53. A kit for detecting at least one target nucleic acid sequence in a sample comprising:

(a) a ligation probe set for each target nucleic acid sequence, the probe set comprising

5 (i) at least one first probe, comprising a target-specific portion, a 5' primer-specific portion, wherein the 5' primer-specific portion comprises a sequence, and

(ii) at least one second probe, comprising a target-specific portion, a 3' primer-specific portion, wherein the 3' primer-specific portion comprises a  
10 sequence,

wherein the probes in each set are suitable for ligation together when hybridized adjacent to one another on a complementary target nucleic acid sequence; and

(b) a buffer comprising poly-deoxy-inosinic-deoxy-cytidylic acid.  
15

54. The kit of claim 53, further comprising at least one primer set comprising (i) at least one first primer comprising the sequence of the 5' primer-specific portion of the at least one first probe, and (ii) at least one second primer comprising a sequence complementary to the sequence of the 3' primer-specific  
20 portion of the at least one second probe.

55. A composition for a ligation reaction comprising a ligase and poly-deoxy-inosinic-deoxy-cytidylic acid.